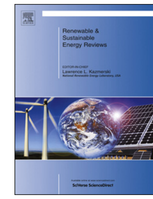
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Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review

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ABSTRACT

Using wastewater as resource for microalgal cultivation was seriously considered as a promising approach for sustainable biomass and lipid production. The proper selection of microalgal species is the foundation and key point to achieve this objective. This paper reviewed the recent status of microalgal cultivation in wastewater, including the characteristics of microalgal species used in recent studies, the performance of different microalgal species in different types of wastewater, the commonly-used isolation methods of microalgal species adaptable to the growth in wastewater, and the evaluation criteria of microalgal species. It was found that microalgal biomass and lipid production in wastewater were comparable to those in artificial culture medium, although most of the data was obtained in sterilized wastewater. Among all microalgal species involved in this review, *Botryococcus braunii*, *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* showed superior performance in certain studies. However, no microalgal species has been demonstrated to meet all the requirements for large-scale biomass production in wastewater. Thus, the efforts on microalgal species isolation and characterization should still be promoted. On the basis of all the information, this review explored the limitations of recent studies and future research needs on this topic.

Contents

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1. Introduction

Nowadays about 80% of global energy demand is provided by fossil fuels. However, this energy form is nonrenewable, and energy crisis has become one of the most important challenges faced by human society in the 21th century. Furthermore, the rapid consumption of fossil fuels has released large amounts of greenhouse gases into the atmosphere, thereby aggravating global climate change [1]. Many countries and researchers are thus focusing on the development of new, clean and sustainable energy sources as substitutes of fossil fuels. Compared with other energy forms, microalgal bioenergy obtained some irreplaceable advantages: (1) high lipid content and rapid growth resulting in higher lipid productivity than that of oilseed crops [2,3]; (2) valuable chemicals production [4]; (3) the possibility to be integrated with CO_2 capture [3,5]; and (4) the perspective to couple energy production with wastewater treatment [6].

Due to the differences in the downstream processes, there are several kinds of products that could be produced from microalgal biomass, as shown in Fig. 1. The triacylglycerols (TAG) in microalgal lipid could be converted into biodiesel via transesterification process [7]. This process is already mature and reliable, and the conversion efficiency could be as high as 96% in certain cases [8]. The saccharides in microalgal biomass could be used to produce bioethanol or biogas via fermentation [9] or anaerobic digestion [10]. Also, some microalgal species could produce biohydrogen via biophotolysis of water [11] or biodegradation of starch [12]. The protein in microalgal biomass is a superior raw material for animal feeds, and could be used as additive of animal feeds or as fish feeds [13]. Besides, some microalgal species could produce several valuable fine chemicals, such as polyunsaturated fatty acids, saccharides which could improve human immunity, astaxanthin, carotenoid etc. [4,14,15].

Although microalgae-based bioenergy is considered renewable and sustainable by many researchers [7,16], the resource consumption during the production of microalgal biomass is inevitable and may become the main barrier in the future large-scale application of this energy form. Several life-cycle assessments have been conducted to analyze the resource demand in the large-scale production of microalgal biofuel. Water and inorganic nutrients were identified as important limiting resources [17–19]. In the assessment of Yang et al., the water, nitrogen and phosphorus usage of microalgal biodiesel under the scale regulated by the Energy Independence and Security Act of the USA would reach 85.7%, 31% and 103.5% of the national usage in 2010 if freshwater is used without recycling [17].

However, the huge consumption of water and nutrients for the production of microalgal biomass could be offset by coupling microalgal cultivation with wastewater treatment [6]. As highlighted by Yang, using wastewater for microalgal cultivation would reduce 90% water demand and eliminate the need of all the nutrients except phosphorus [17]. Besides the inorganic nutrients, part of the organic matters in wastewater could also be utilized for the mixotrophic cultivation of some microalgae. The biomass and lipid production of microalgae under mixotrophic growth conditions was usually much higher than that under photoautotrophic growth conditions [20–22]. Therefore, wastewater could provide most essential resources for large-scale microalgal cultivation, including water resource, organic matter and inorganic nutrients (as shown in Fig. 2).

The technology of utilizing microalgae for low-cost and environment-friendly wastewater treatment process as well as an alternative energy source was analyzed early on [23,24]. But the possibility to achieve dual purpose in a single coupled system has only been recently proposed [6,16]. Because of the promising sustainability of microalgal cultivation using wastewater as resource, many researchers have been focusing on this topic.

Microalgae could grow in various living conditions, even in some extreme conditions; for example, *Dunaliella salina* which grows in high salinity [25]. However, due to the particularities and complexity of wastewater, many microalgal species are not adapted to wastewater. In the research of Li et al., 8 microalgal species among 12 species, which were highlighted in the literature because of high biomass production and high lipid content, showed nearly no growth in domestic secondary effluent [26]. These results indicate that specific microalgal species could only adapt to specific growth environment. Therefore, the proper selection of microalgal species is

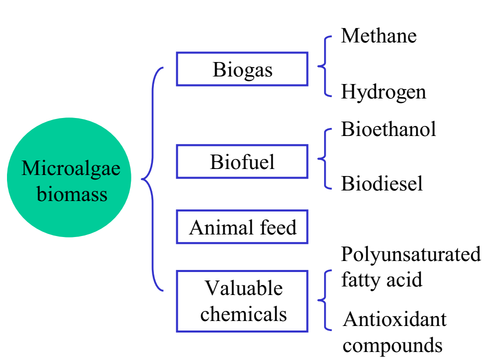


Fig. 1. The products could be obtained from microalgal biomass.

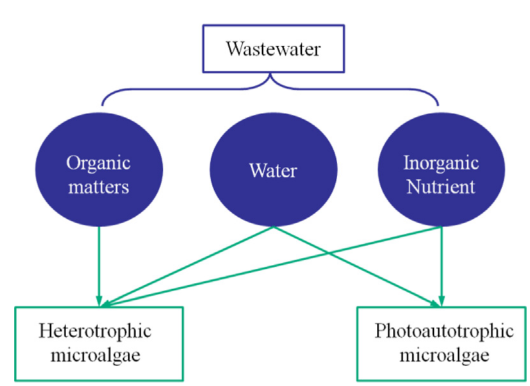


Fig. 2. Resources within wastewater for microalgal cultivation.

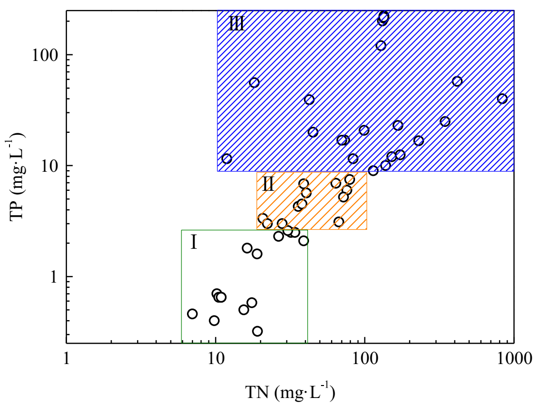


Fig. 3. The TN and TP concentrations of wastewater used in recent researches. Region-I represents domestic secondary effluent; Region-II represents domestic wastewater or domestic primary effluent; Region-III represents wastewater from livestock breeding and agriculture or centrate of domestic wastewater.

the foundation and key point to achieve sustainable microalgal biomass/lipid production using wastewater as resource.

In this review, the recent efforts on microalgal cultivation using wastewater as resource are summarized. The literature cited by this review was found in Web of Knowledge. Most of the literature published around 2007 to 2013 with the words "microalga*" and "wastewater" in the titles or abstracts or key words were included.

Generally, this review summarizes the characteristics of wastewater used in recent studies, the most commonly-used microalgal species and part of their cellular properties, the biomass and lipid production of different microalgal species in wastewater, the isolation approaches of microalgal species and the selecting indices of microalgal species adaptable to the growth in wastewater. On this basis, the review also explores the limitations of recent studies and future research needs for sustainable microalgal biomass/lipid production using wastewater as resource.

2. Characteristics of wastewater used in recent researches for microalgal cultivation

The total nitrogen (TN) and total phosphorus (TP) concentrations of wastewater would differ significantly depending on the wastewater type. Fig. 3 summarizes the TN and TP concentrations of wastewater used in recent researches for microalgal cultivation. This figure can be divided into three regions representing different types of wastewater. In Region-I, the concentrations of TN and TP are relatively low (TN: about 5–30 mg L^{-1}; TP: about 0.2–3 mg L^{-1}), and it is the typical water quality of domestic secondary effluent. In Region-II, the concentrations of TN and TP are in the range of 20–80 mg L^{-1} and 3–7 mg L^{-1}, representing domestic wastewater or domestic primary effluent. In Region-III, the concentrations of TN and TP are much higher (TN: about 10–1000 mg L^{-1}; TP: about 9–110 mg L^{-1}) than that in the other two regions. The wastewater from livestock breeding and agriculture is usually in this region, such as anaerobic digested poultry litter effluent or dairy manure and olive-oil mill wastewater. However, these kinds of wastewater always contained nutrients of an extremely high concentration, and thus had to be diluted before microalgal cultivation. Besides, the centrate of domestic wastewater was also in Region-III.

Although wastewater could provide some essential resources for large-scale microalgal cultivation, it is quite different from common culture medium. The major difference between wastewater and artificial culture medium is the high complexity of wastewater in terms of composition. The concentration of nutrients in wastewater, such as nitrogen and phosphorus, varies in a large range (as shown in Fig. 3). Much nitrogen may exist in wastewater in the form of ammonia, which could inhibit microalgal growth at high concentration [27,28]. Besides the high concentration of ammonia, wastewater could also contain other chemical or biotic inhibitor of microalgal growth. In domestic wastewater, the organic matters could stimulate the growth of other microorganisms such as bacteria, so that they may outcompete microalgae for the uptake of essential nutrients. While in industrial-derived wastewater, the presence of toxins such as heavy metals (cadmium, mercury or zinc) is an important factor which can interfere with microalgal growth [29]. In addition, the pathogenic bacteria and virus or predatory zooplankton in different kinds of wastewater could also inhibit microalgal growth as well [5]. These adverse factors will differ significantly depending on the wastewater type or from one wastewater treatment site to another, and set higher requirements on microalgal species than that in traditional microalgal cultivation.

3. Microalgal species used in recent researches

Microalgal species used in recent researches and some of their cellular properties are summarized in Table 1. The main downstream products of these microalgae are microalgal lipid and biodiesel. Generally, the microalgae cultivated within wastewater could be divided into photoautotrophic microalgae and mixotrophic microalgae according to the carbon source used by microalgal cell. Photoautotrophic microalgae could only assimilate inorganic carbon for growth and showed nearly no removal to the organic carbon in wastewater; while mixotrophic microalgae could assimilate both inorganic carbon and organic carbon (usually determined by Chemical Oxygen Demand, COD) in wastewater. Because the organic matters varied widely in different types of wastewater, some microalgal species would show the ability of photoautotrophic growth in a specific wastewater, and the ability of mixotrophic growth in another. These microalgal species include *Botryococcus braunii*, *Chlorella vuglaris*, *Scenedesmus obliquus*, etc.

Among photoautotrophic microalgal species, unicellular green microalgae seems to be particularly tolerant to many wastewater conditions [22,26,30]. Therefore, they are the most commonly-used microalgal species. As shown in Table 1, there are 25 green microalgal species out of all the 35 species. Among green microalgae, *Chlorella* and *Scenedesmus* are usually the predominant species of the microalgal communities in waste stabilization ponds [31,32] or high-rate algal ponds [33].

Although microalgae used to be defined as a kind of photoautotrophic microorganism, their ability of assimilating organic carbon has been demonstrated in recent researches [34]. Furthermore, the biomass and lipid production of some microalgae were enhanced significantly when certain organic matters were used as carbon source [35]. There are several kinds of carbon matters within wastewater which could be utilized for mixotrophic microalgal cultivation. In the research of Markou et al., *Sprirulina platensis* was found to be able to assimilate phenols and carbohydrate in the olive-oil mill wastewater, and a total chemical oxygen demand (COD) removal of 73.18% was achieved [36]. Besides *Sprirulina platensis*, several other microalgal species were found to obtain the ability of mixotrophic growth in wastewater, including *Botryococcus braunii*, *Chlorella minutissima*, *Scenedesmus obliquus* etc. All the microalgal species mentioned above showed considerable COD removal property.

The size and shape of different microalgal species used in recent studies are various, and these properties are highly related to the ability of settlement. Gravitational settling is one of the most commonly used and simple approaches for microalgal biomass harvest [37]. According to Stokes' law, the settling rate

Table 1
Microalgal species used for bioenergy production using wastewater in recent researches.

Microalgal species	Cell shape and size (μm)	Wastewater type	Reference
Photoautotrophic microalgal species			
Botryococcus braunii	Ellipsoid, cluster; *L*: 10–15; *W*: 3–7	Secondarily treated piggery wastewater; carpet industry effluents; domestic secondary effluent	[26,30,50,97]
Chlamydomonas debaryana	Ellipsoid; *R*: 6–10	Domestic secondary effluent	[22]
Chlamydomonas pitschmannii	Ellipsoid; *R*: 6–10	Domestic secondary effluent	[22]
Chlamydomonas reinhardtii	Ellipsoid; *R*: 6–10	Influent, effluent and centrate of municipal wastewater	[51]
Chlorella ellipsoidea	Ellipsoid; *R*: 2–6	Domestic secondary effluent	[54]
Chlorella minutissima	Sphere; *R*: 2–6	Municipal wastewater	[30]
Chlorella saccharophila	Sphere; *R*: 2–6	Carpet industry effluents	[97]
Chlorella sorokiniana	Sphere; *R*: 2–6	Domestic secondary effluent	[26]
Chlorella stigmatophora	Sphere; *R*: 2–6	Domestic secondary effluent	[104]
Chlorella vuglaris	Sphere; *R*: 2–6	Domestic secondary effluent; municipal wastewater;	[22,26,30,105,106,107,108, 109,43,44,110]
Chlorella sp.	Sphere; *R*: 2–6	Municipal wastewater	[111]
Cyclotella hebeiana	Disc-shaped; *R*: 15–50	Domestic secondary effluent	[26]
Dunaliella primolecta	Ellipsoid; *R*: 10–16	Domestic secondary effluent	[26]
Dunaliella tertiolecta	Ellipsoid; *R*: 10–16	Carpet industry effluents	[97]
Isochrysis sp.	Ellipsoid; *L*: 4.4–7.1; *W*: 2.7–4.4	Domestic secondary effluent	[26]
Micractinium sp.	Ellipsoid; *R*: 5–9	Domestic secondary effluent	[22]
Neochloris oleoabundans	Sphere; *R*: 4–8	Domestic secondary effluent	[48]
Nitzschia hantzschiana	Cylinder/rhomb; *L*: 8–12; *W*: 2–4	Domestic secondary effluent	[26]
Parachlorella (*Chlorella*) *kessleri*	Sphere; *R*: 2–6	Oil sands tailings pond water	[112]
Phaeodactytum ericornutum	Fusiformis; *L*: 8–12; *W*: 3–4	Domestic secondary effluent	[26]
Planktothrix isothrix	Strip; *L*: 100–200; *W*: 2–4	Municipal wastewater	[44]
Pleurochrysis carterae	Sphere/ellipsoid; *R*: 1–4	Carpet industry effluents	[97]
Scenedesmus dimorphus	Tetramer; *L*: 4–10; *W*: 3–7	Secondary effluent of agroindustrial wastewater	[107]
Scenedesmus obliquus	Tetramer; *L*: 4–10; *W*: 3–7	Domestic secondary effluent	[108,113]
Scenedesmus quadricauda	Tetramer; *L*: 4–10; *W*: 3–7	Municipal wastewater	[114]
Scenedesmus rubescens	Tetramer; *L*: 4–10; *W*: 3–7	Municipal wastewater	[109]
Scenedesmus sp.	Tetramer; *L*: 4–10; *W*: 3–7	Municipal wastewater; domestic secondary effluent	[22,26]
Schizochytrium sp.	Sphere; *R*: 2–8	Domestic secondary effluent	[26]
Spirulina platensis	Spiral; *L*: 200–500; *W*: 5–10	Domestic secondary effluent	[26]
Synechococcus nidulans	Ellipsoid; *R*: 0.8–1.5	Domestic secondary effluent	[30]
Mixotrophic microalgal species			
Auxenochlorella protothecoides	Ellipsoid	Concentrated municipal wastewater	[49,55]
Botryococcus braunii	Ellipsoid, cluster; *L*: 10–15; *W*: 3–7	Domestic secondary effluent	[115]
Chlorella kessleri	Sphere; *R*: 2–6	Domestic secondary effluent	[116]
Chlorella minutissima	Sphere; *R*: 2–6	Municipal wastewater	[117]
Chlorella pyrenoidosa	Sphere; *R*: 2–6	Soybean processing wastewater; biogas wastewater	[53,118]
Chlorella sorokiniana	Sphere; *R*: 2–6	Diluted anaerobically digested poultry litter effluent; concentrated municipal wastewater; domestic secondary effluent; pretreated anaerobically digested sludge liquor	[22,55,117,119]
Chlorella vuglaris	Sphere; *R*: 2–6	Industrial dairy waste; concentrated municipal wastewater	[55,20]
Heynigia sp.	–	Concentrated municipal wastewater	[55]
Hindakia sp.	–	Concentrated municipal wastewater	[55]
Micractinium sp.	Ellipsoid; *R*: 5–9	Concentrated municipal wastewater	[55]
Scenedesmus bijuga	Tetramer; *L*: 4–10; *W*: 3–7	Diluted anaerobically digested poultry litter effluent	[117]
Scenedesmus obliquus	Tetramer; *L*: 4–10; *W*: 3–7	Brewery effluent	[120]
Scenedesmus sp.	Tetramer; *L*: 4–10; *W*: 3–7	Concentrated municipal wastewater	[55]
Spirulina platensis	Spiral; *L*: 200–500; *W*: 5–10	Diluted olive-oil mill wastewater	[36]

Part of the information about the cell shape and size was from the Culture Collection of Algae at The University of Texas at Austin and Microbial Culture Collection at National Institute for Environmental Studies. *L*=length; *W*=width; *R*=radius.

of particles would increase significantly with the increase of the equivalent radius, and thus the microalgal cell with larger cell size would be easier to settle. The radius of *Chlorella* is in the range of 2–6 μm (usually less than 4 μm), which was relatively small among all the commonly-used microalgal species leading to the difficulty in settling.

Besides the size of a single cell, the morphology of microalgal cell would also be another important factor influencing the settling rate, because some microalgal species would form aggregation with much larger size than a single cell during growth naturally increasing the settling ability significantly. For example, *Botryococcus braunii* would form clusters of several cells during growth while *Scenedesmus* would usually grow in the way of tetramer. These particular aggregations formed by different microalgal species could also protect themselves from the predators in wastewater to some extent.

The performance on biomass production and lipid accumulation of different microalgal species varies significantly in wastewater. In the next section, the general biomass and lipid production properties of these microalgal species will be reviewed briefly.

4. Biomass and lipid production of different microalgal species in wastewater

In the recent studies on microalgal biomass and lipid production in wastewater, researchers usually focused only on microalgal cultivation without the discussion on the downstream process of biomass. One of the main reasons is that the downstream process is relatively mature, and there is no essential difference between the conversion of microalgal biomass from wastewater and biomass from other sources. Besides, the downstream process might be beyond the research area of some researchers focusing on microalgal cultivation.

In this section, the biomass and lipid production properties of microalgal species listed in Table 1 are summarized, and the downstream process of biomass to produce bioenergy is discussed subsequently. Generally, the biomass and lipid production of microalgae would be influenced significantly by nutrient level [38,39], temperature [38,40] and light [41,42]. However, the research on these influence fators are always conducted in artificial culture medium rather than in wastewater. Some researchers investigated the effects of different nutrient levels in wastewater on microalgal biomass and lipid production by adding extra nutrient into wastewater [43,44]. But, as for temperature and light, only normal cultivation conditions were used in these studies (temperature: around 25–30 °C, light: about 40–200 μmol photons m^{-2} s^{-1}).

4.1. Biomass production

The maximal biomass production of some microalgal species in wastewater are summarized in Fig. 4. As the nitrogen and phosphorus concentrations of different wastewater varied widely (as shown in Fig. 3), the biomass production of a single microalgal species changed in a wide range. Therefore, a simple average value could not represent the growth condition of a specific microalgal species in wastewater. The data points were divided into three types just as that in Fig. 3, representing the biomass production of a specific microalgal species in a specific wastewater region (Fig. 4). In addition, Fig. 4 is divided into two parts: the left part summarizes the biomass production obtained in photoautotrophic growth; the right part summarizes those in mixotrophic growth.

When the nutrient concentration, especially nitrogen and phosphorus, increased in a certain range, the microalgal biomass production would increase significantly [45–47]. Thus, microalgal biomass production obtained in the wastewater Region-I was usually much lower (<0.6 g L^{-1}) than that obtained in other two regions. However, in some researchers' studies, some microalgal species could also achieve relatively high biomass production at low nutrient concentration. A typical example is that *Botryococcus braunii* obtained a biomass production as high as 1.88 g L^{-1} in domestic secondary effluent (NH_4^+: 12.76 mg L^{-1}; PO_4^{3-}: 2.0 mg L^{-1}) in an 11 l BioFlo reactor at 25 °C and 3500 lx in 12:12 h (light:dark) period [30].

In wastewater Region-II, microalgal biomass production varied from 0.2 to 2.1 g L^{-1}. Compared with other microalgal species, *Neochloris oleoabundans* obtained higher biomass production. In the study of Wang et al., *Neochloris oleoabundans* was cultivated in secondary municipal wastewater with the addition of extra nitrogen and phosphorus [48]. A biomass production of 2.1 g L^{-1} was achieved with the enrichment of 70 mg N L^{-1} at 30 °C.

In wastewater Region-III, many microalgal species showed the ability to remove COD from wastewater, and thus they were considered in the condition of mixotrophic growth, such as *Auxenochlorella protothecoides* [49] and *Spirulina platensis* [36]. Due to higher nitrogen and phosphorus concentration in wastewater Region-III, most microalgal species obtained higher biomass production, ranging from 0.3 to 8.5 g L^{-1}. Interestingly, the highest biomass production in Region-III was achieved in photoautotrophic growth rather than mixotrophic growth. In the study of An et al., *Botryococcus braunii* was cultivated in secondarily treated piggery wastewater with a TN concentration of 836 mg L^{-1} and a PO_4–P concentration of 40 mg L^{-1}, and a biomass production as high as 8.5 g L^{-1} was achieved after 12 d at 25 °C and continuous illumination of 100 μmol photons m^{-2} s^{-1} [50]. However, this study did not reveal the ability of *Botryococcus braunii* using the organic matters in secondarily treated piggery wastewater [50]. Similar biomass production (about 8.2 g L^{-1}) was achieved in the study of Kong et al. by *Chlamydomonas reinhardtii* in 100% centrate of municipal wastewater with TKN concentration of 128.6 mg L^{-1} and TP concentration of 120.6 mg L^{-1} at 25 °C and continuous illumination of 200 μmol photons m^{-2} s^{-1} [51].

The maximal biomass production was a parameter related to cultivation time to some extent. In order to better cross-reference the data in literature, the average biomass productivity, that is, the maximal biomass production divided by cultivation time, was cited or calculated and summarized in Fig. 5.

Most microalgal biomass productivity was below 0.2 g L^{-1} d^{-1} (about 70% of all the data points), which was similar to the biomass productivity in artificial culture medium as reviewed by Griffiths et al. [52]. However, extremely high biomass productivity was also achieved by some microalgal species in certain wastewater. *Botryococcus braunii* in secondary treated piggery wastewater was a prime example as highlighted above with an average biomass productivity about 0.72 g L^{-1} d^{-1} [50].

Besides *Botryococcus braunii*, *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* also obtained high biomass productivity in different studies. In the study of Su et al., *Chlorella pyrenoidosa* was cultivated in soybean processing wastewater and an average biomass productivity of 0.64 g L^{-1} d^{-1} was achieved by fed-batch culture [53]; in the study of Kong et al., *Chlamydomonas reinhardtii* was cultivated in centrate of municipal wastewater and a maximal biomass productivity of 2.0 g L^{-1} d^{-1} was achieved [51].

These values of microalgal biomass productivity in wastewater were even higher than the maximal value achieved in artificial medium as reviewed by Griffiths [52], that is, 0.59 g L^{-1} d^{-1} obtained by *Tetrashlmis suecica*. These results demonstrated the potential of using wastewater as resource for microalgal cultivation.

4.2. Lipid production

The lipid content of different microalgal species cultivated in wastewater is summarized in Fig. 6. In many studies, the lipid content of microalgae was not determined. Thus, the data size of Fig. 6 was

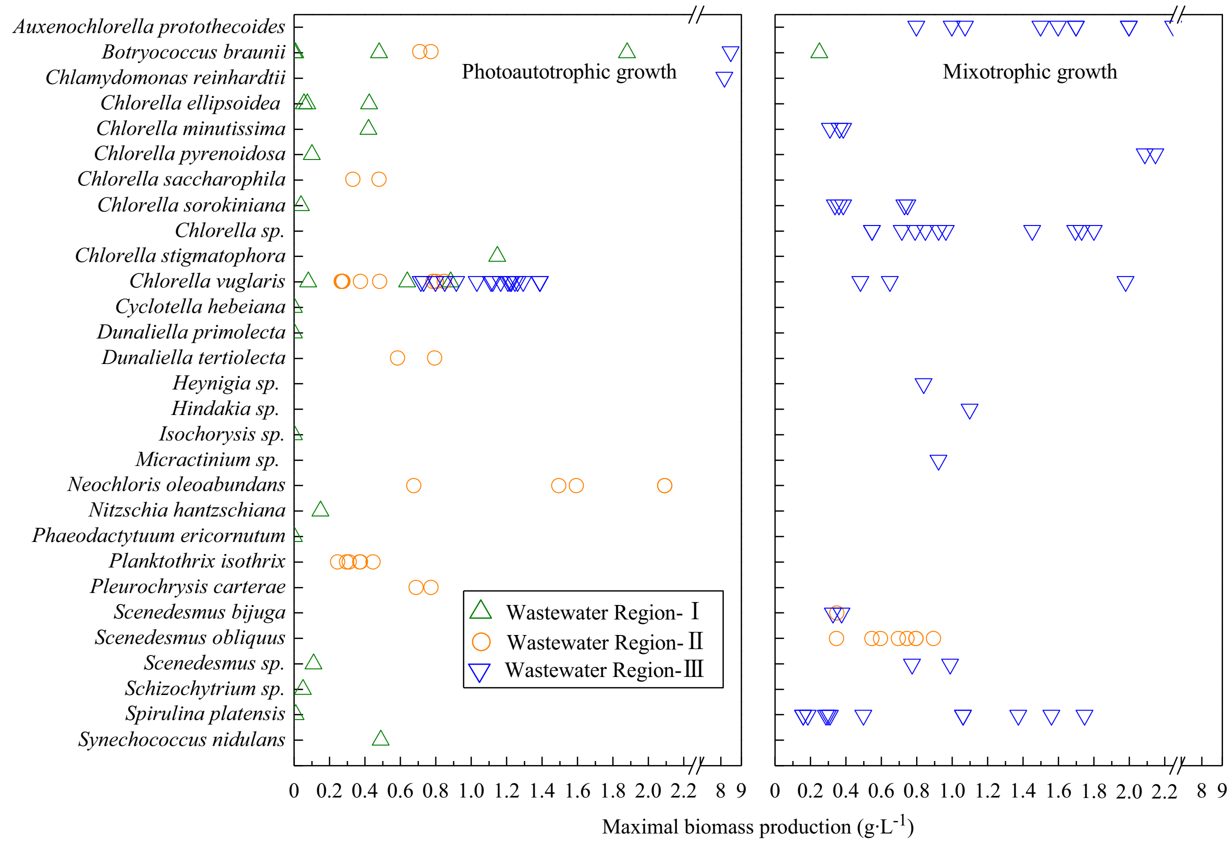


Fig. 4. Maximal biomass production of different microalgal species in wastewater.

obviously smaller than that of Figs. 3 and 4. The lipid content of most microalgae was in the range of 10–30%. Microalgal lipid accumulation was usually enhanced by nutrient depletion [38,45]. Therefore, in contrast to biomass production and productivity, the highest lipid content was achieved in domestic secondary effluent with low nutrient concentration. *Scenedesmus* sp. LX1 was a promising microalgal species in domestic secondary effluent, with better performance on biomass production and lipid accumulation than other 11 high-lipid-content microalgae [26]. In addition, *Chlorella ellipsoidea* YJ1 isolated by Yang could achieve a lipid content of about 40% in domestic secondary effluent [54], which is the highest value in Fig. 6.

In the previous studies, microalgal biomass production was usually found to contradict lipid content, as microalgal biomass production was inhibited by nutrient depletion while the lipid content was enhanced under the same cultivation condition. Thus, when the lipid content was highest, the total lipid production was not necessarily high [38,45]. Griffiths et al. proposed lipid productivity as a key index for lipid production by microalgae [52]. In the review conducted by Griffiths et al., the average lipid productivity was calculated as the product of average microalgal biomass productivity and lipid content. In this review, the same method was used to obtain the average lipid productivity if this value was not given in the literature. These data (cited and calculated) are summarized in Fig. 7.

Generally, the average lipid productivity of all the microalgal species in wastewater Regions-I and II was less than 10 mg L^{-1} d^{-1}. Compared with that, many microalgal species in Wastewater Region-III could achieve average lipid productivity ranging from 20 to 100 mg L^{-1} d^{-1}. The lipid productivity of *Auxenochlorella protothecoides* [49], *Chlamydomonas debaryana* [51], *Chlorella sorokiniana* [55] and *Hindakia* sp. [55] in Region-III could reach about 80 mg L^{-1} d^{-1} or even higher.

Furthermore, the average lipid productivity of two other microalgal species was more than 200 mg L^{-1} d^{-1}, which was even higher than the maximal value achieved in artificial medium as reviewed by Griffiths et al. [52]. In the study of Su et al., *Chlorella pyrenoidosa* achieved an average lipid productivity of 236.8 mg L^{-1} d^{-1} under the condition of fed-batch culture in soybean processing wastewater at 27 ± 1 °C and 40.5 μmol photons m^{-2} s^{-1} in 14:10 h (light:dark) period [53]. In the study of Abreu et al., *Chlorella vuglaris* was cultivated in hydrolyzed cheese whey (5 g L^{-1} glucose and 5 g L^{-1} galactose) at 30 °C and 70 μmol photons m^{-2} s^{-1}, and a lipid productivity about 250 mg L^{-1} d^{-1} was obtained [20].

The average lipid productivity seemed to be more closely related to the average biomass productivity rather than lipid content, mainly because the lipid content would only change in a limited range (from 10% to 40% as shown in Fig. 6), but the biomass productivity would change much more significantly. Thus, higher lipid productivity was usually achieved along with higher biomass productivity. Similar phenomenon was also found in the study of Griffiths et al. [52].

In summary, microalgal biomass and lipid production obtained from wastewater was comparable to that obtained from artificial culture medium. In some cases, the biomass and lipid productivity of certain microalgal species was even higher than the maximal value achieved in artificial culture medium as reviewed by Griffiths et al. The biomass and lipid productivity of different microalgal species increased generally with the increase of the nitrogen and phosphorus concentration in wastewater (from wastewater Region-I to Region-III). However, some microalgal species also achieved high biomass production under relatively low nutrient concentration, such as *Botryococcus braunii* in the study of Sydney et al. Among all the microalgal species involved in this review, *Botryococcus braunii*, *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* showed superior

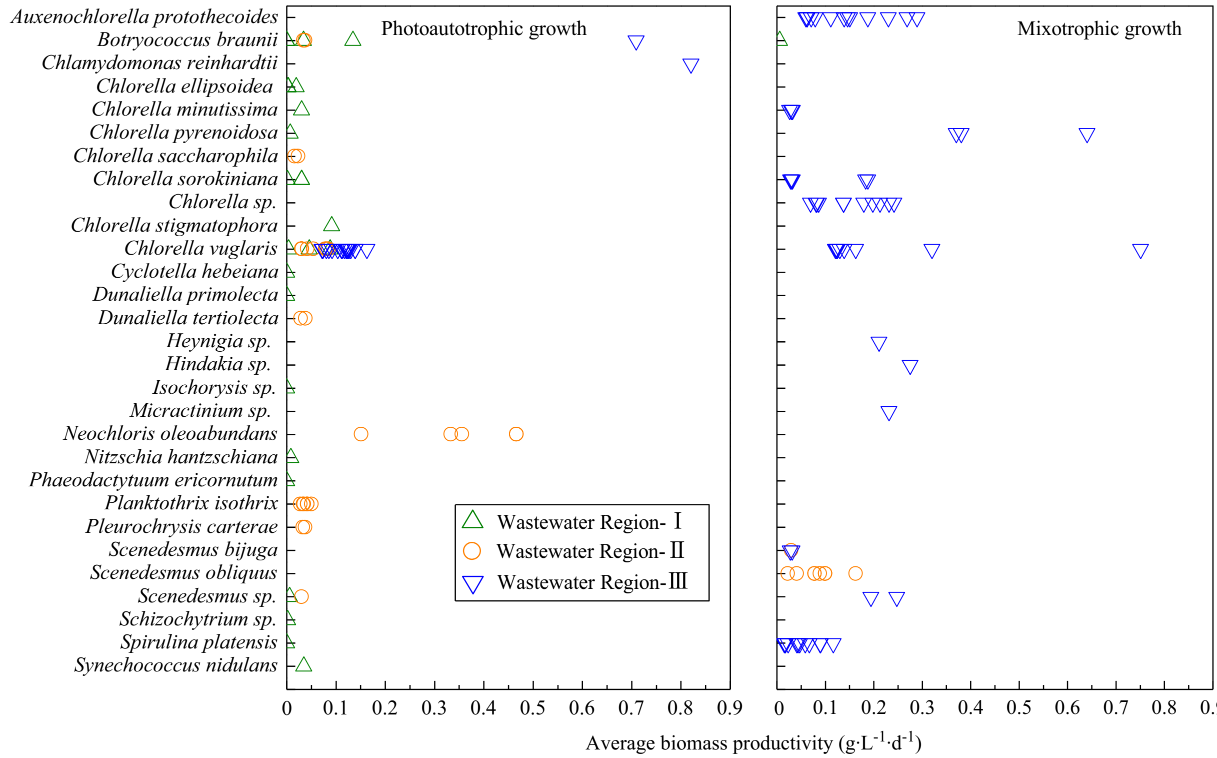


Fig. 5. Average biomass productivity of different microalgal species in wastewater.

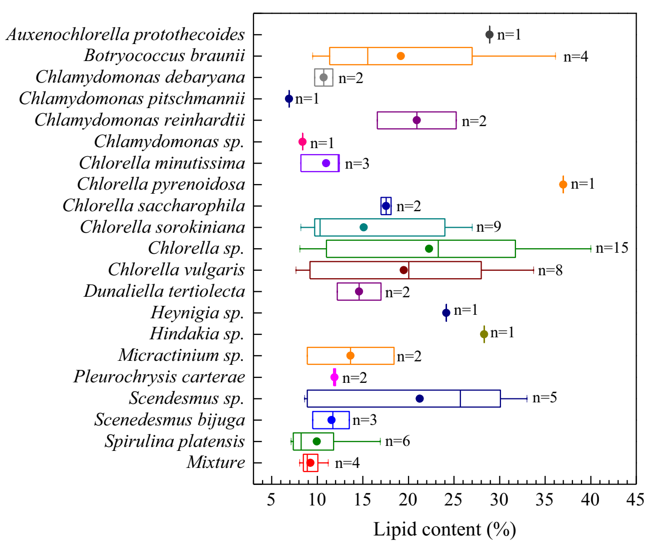


Fig. 6. Lipid content of different microalgal species in wastewater.

performance on biomass production or lipid production in certain wastewater.

4.3. The downstream process converting microalgal biomass to bioenergy

Several potential downstream processes exist for the conversion from microalgal biomass to different kinds of bioenergy. These downstream processes and corresponding products are summarized in Table 2. Generally, the processes converting microalgal biomass can be classified into three categories: (1) the technologies processing whole microalgal biomass; (2) those processing microalgal extracts (e.g., lipids, carbohydrates); and (3) those processing microalgal remnants after extraction. Most of these technologies are primarily based on similar methods developed for the conversion of terrestrial plant-based oils and other products into bioenergy, although the compositional complexities of the output streams from microalgae must be dealt with before these technologies can be applied effectively [5].

The pros and cons of these technologies within each of the three categories have already been reviewed in detail in National Algal Biofuels Technology Roadmap of the U.S. Department of Energy [5]. Among all the technologies, those processing microalgal extracts are relatively mature and economical. The conversion efficiency of chemical transesterification can be as high as 96% under optimized conditions [8], and this is why biodiesel is considered as the potential product of microalgal biomass in most literature about microalgal cultivation in wastewater. The enzymatic conversion process is similar to chemical transesterification, except that biocatalysts (lipases) are used [56]. The catalytic cracking technology has already provided the renewable jet fuel blends (derived from lipids obtained from jatropha and microalgae) used in recent commercial jet test flights [5].

Compared with the technologies above, anaerobic digestion and fermentation is also relatively mature, and has already been used to process whole microalgal biomass [5,57] and microalgal remnants after extraction [5,10]. Other technologies processing whole biomass, such as supercritical fluids conversion [58], pyrolysis [59], liquefaction [60], gasification [61], are mainly used to convert biomass from terrestrial plants, rather than microalgal biomass. The application of these technologies after optimization in processing microalgal biomass is conducted mainly in laboratory scale.

The processes converting microalgal biomass from wastewater would not be significantly different from those converting biomass from artificial culture medium. However, it is necessary to

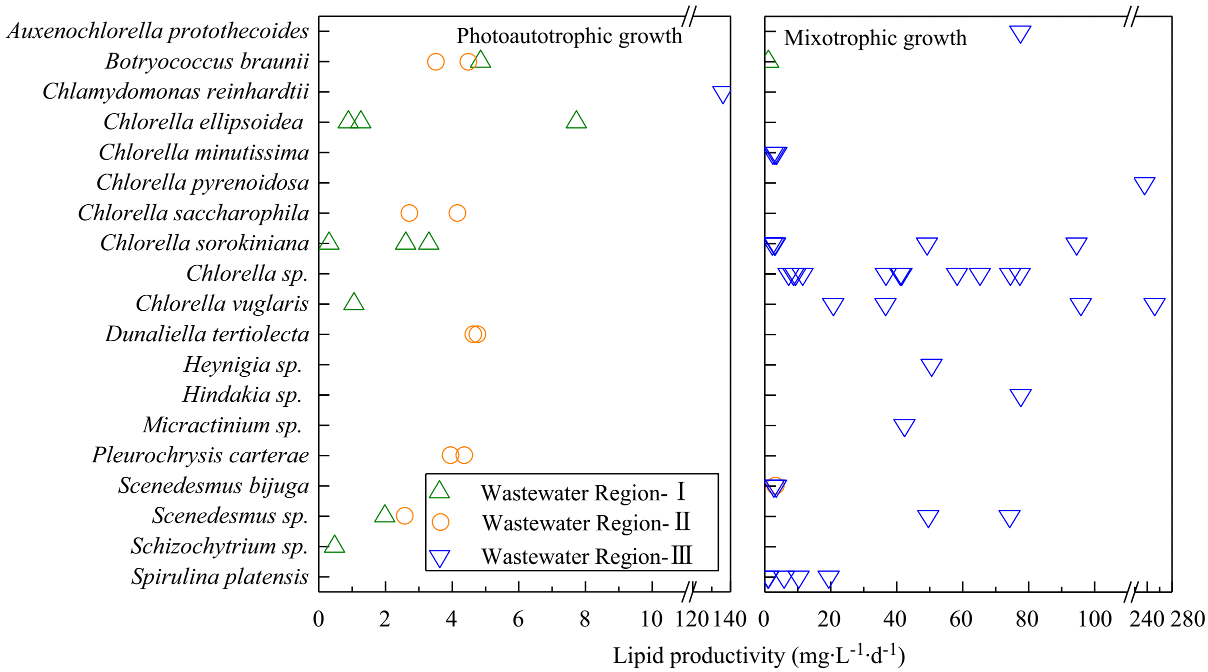


Fig. 7. Average lipid productivity of different microalgal species in wastewater.

consider the possible risk of processing microalgal biomass from wastewater. Many microalgal species have strong ability to adsorb heavy metals, such as cadmium, lead [62], nickel, zinc [63], etc. Besides heavy metals, some micropollutants such as Persistent Organic Pollutants (POPs) and Pharmaceutical and Personal Care Products (PPCPs) might also accumulate in microalgal biomass [64–66]. These pollutants, not surprisingly, would bring along uncertain risk and adverse effects on the conversion processes. Nevertheless, there have been few relevant researches.

5. Isolation methods of microalgal species adaptable to the growth in wastewater

In order to obtain microalgal species adaptable to wastewater, researchers always collected samples directly from wastewater or from the environment similar to wastewater. The general isolation process of microalgal species is shown in Fig. 8.

Most of the data showed in Figs. 4–7 are from the microalgal species isolated in the researchers' studies. These species usually showed better performance in wastewater than those from other environment. For example, in the research of Li et al., *Scenedesmus* sp. LX1 showed higher biomass production, nutrient removal efficiency and lipid content than other 11 microalgal species reported in literature when cultivated in domestic secondary effluent [26]. One of the most important reasons for this phenomenon was that *Scenedesmus* sp. LX1 was isolated from tap water which contained nutrient at an extremely low concentration. Therefore, this microalgal species could adapt to domestic secondary effluent with a nutrient concentration much lower than that in artificial culture medium while other microalgal species could not. Similar examples could be found in other studies [22,30]. Generally, municipal wastewater, domestic secondary effluent and the walls of aeration tank in wastewater treatment plant could be the sample source for microalgal isolation.

However, in the isolation process, the consistency between local growth conditions and laboratory cultivation conditions is very difficult to maintain. In order to increase microalgal density within water sample or the growth rate of algal colony on the agar plate,

Table 2
The downstream process of microalgal biomass to produce bioenergy.

Downstream process	Products	Reference
Technologies processing whole microalgal biomass		
Anaerobic digestion	Biogas	[5,57]
Supercritical fluids conversion	Liquid/gas fuels	[58]
Pyrolysis	Liquid/gas fuels	[59]
Liquefaction	Liquid fuels	[60]
Gasification	Hydrogen	[5]
	Liquid hydrogen fuels	[61]
Gasification-higher alcohol synthesis	Methanol, ethanol, etc.	[121]
Technologies processing microalgal extracts (e.g., lipids, carbohydrates)		
Chemical transesterification	Biodiesel	[16,122]
Enzymatic conversion	Biodiesel	[56]
Catalytic cracking	Gasoline, kerosene, diesel, olefin aromatics	[5]
Technologies processing microalgal remnants after extraction		
Anaerobic digestion	Biogas	[10]
Fermentation	Ethanol	[5]

extra inorganic nitrogen and phosphorus is usually added. This is the process of enrichment, leading to different growth conditions from sample source. Therefore, microalgal species obtained after enrichment may not adapt to the original environment from which it was isolated. Technically, enrichment of samples is not indispensable in the process of isolation. Nevertheless, this approach could reduce the difficulty of isolation and the afford a benefit of time saving.

Typically, the isolation methods of microalgal species can be divided into two groups: manual isolation methods and automatic isolation methods. Manual isolation methods are usually based on considerable experimental skills and experience, including traditional isolation methods such as streak plate method, single-cell isolation by micropipette, serial dilution techniques [67] and some novel isolation methods such as micromanipulation [68]. Compared with that, automatic isolation methods are based on microfabricated devices, including atomized cell spray technique [67] and flow cytometer with the capabilities of fluorescent activated cell sorting (FACS) [69,70].

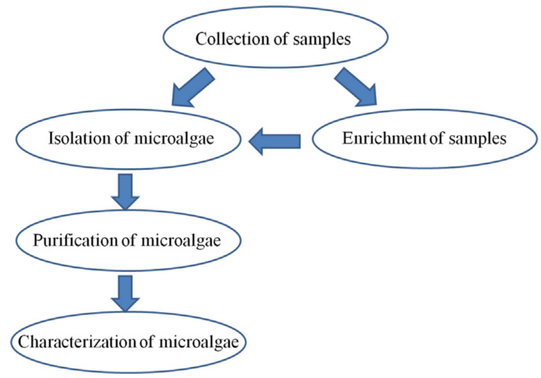


Fig. 8. The general isolation process of microalgal species.

All these isolation methods could be coupled with some reinforcement techniques, including immunological technique and non-immunological techniques [71]. Immunological technique utilizes the specific immunoreactions of cell membrane protein with the capturing antibodies because the integrated proteins are specific for their functions. This method obtains considerable specificity and selectivity but may damage microalgal cell to some extent. On the other hand, non-immunological techniques achieve isolation based on the size, shape and other physical properties of microalgal cell. Different physical forces are usually utilized in these methods, including dielectrophoresis [72], hydrodynamic separation [73] and ultrasound separation [74]. The specificity and selectivity of non-immunological techniques is much lower than immunological technique as cells do not show remarkable differences between each cell type with the exception of immunological properties [71].

The classification and advantages/disadvantages of the isolation methods mentioned above are compared in Table 3. Among all the isolation methods, the technique based on flow cytometer with the capabilities of fluorescent activated cell sorting (FACS) is relatively rapid and precise. More importantly, this method can be applied directly to natural samples without enrichment and thus avoids the change of microalgal living conditions in the isolation process. Therefore, this method seems to be more applicable to the isolation of microalgal species from wastewater samples. The indices of microalgal species characterization will be briefly reviewed in next chapter.

6. Indices to select proper microalgal species

In order to select proper microalgal species, some selecting criteria are needed to distinguish superior microalgal species from others obtained in the isolation process. The selecting criteria are composed of several kinds of indices.

The indices of microalgal species for biomass/lipid production using wastewater as resource are summarized in Table 4. The whole evaluation system of microalgal species was classified into five groups of indices. The indices about microalgal biomass/lipid production and pollutant removal were the most commonly used in the previous species screening. However, due to the particularity and complexity of wastewater, the indices about microalgal resistance to biotic pollution have to be taken into consideration. Also, because of the huge production scale in the future, the indices related to resource consumption are very important.

6.1. Indices about microalgal biomass production

The most direct index about microalgal growth is the maximal biomass production within a certain cultivation time under the same cultivation conditions. This is usually the prime index in the microalgal species screening in wastewater conducted by several research groups [22,26,30]. Besides the final biomass production at the end of cultivation, the growth rate is also very important. There are several indices related to microalgal growth rate, including the specific growth rate (μ), doubling time (t_d) and biomass productivity. According to the definition, the specific growth rate is related to doubling time directly via Eq. (1).

$$\mu = \ln 2/t_d \tag{1}$$

where μ is the specific growth rate and t_d is the doubling time.

Compared with specific growth rate and doubling time, biomass productivity is more closely related to the total biomass production. Therefore, this index is more commonly-used in recent researches [52]. As the covering area of microalgal cultivation pond is limited by the land area available, the studies on the microalgal biomass productivity per unit area presents further practical significance than that on biomass productivity per unit volume [75]. However, this index was usually reported by only one form (either per unit area or per unit volume) in literature, leading to the difficulty in cross-referencing these data. Thus, it is recommended to describe productivity in both ways (per unit area and per unit volume) in further research.

6.2. Indices about microalgal lipid production

Lipid content is the most commonly used index representing the lipid accumulation property of certain microalgal species. However, this index was found not to be the most relevant parameter on the total lipid production both in this review and previous study [52]. The examples that the highest lipid content could not be obtained along with the highest lipid production were very common in microalgal studies [38,45]. Thus, the total lipid production and lipid productivity present further practical significance in the aspect of lipid production.

As microalgal lipid is mainly used as the raw material of biodiesel, the indices related the quality of biodiesel is also very important in the evaluation of microalgal lipid accumulation property. These indices usually include the triacylglycerol (TAG) content in microalgal lipid, the fatty acid (FA) composition and the total fatty acid methyl ester (FAME) content [76,77]. TAG is the direct material for biodiesel production; FA composition would influence the quality of biodiesel produced from microalgal lipid; FAME is actually the main content of biodiesel. Only parts of these indices were used in recent researches. However, the applicability of a certain microalgal species as the feedstock for biodiesel production can be evaluated comprehensively only if all these indices are determined.

6.3. Indices about pollutant removal

The evaluation of nutrient removal property is relatively easier compared with growth and lipid accumulation. The removal efficiency of nitrogen and phosphorus within certain cultivation time is the most commonly-used index. In recent researches, the abilities of different microalgal species assimilating organic matters in wastewater had been revealed [22,36]. Thus, the removal efficiency of chemical oxygen demand (COD) was also used by some researchers to evaluate the nutrient removal property of microalgae [49]. Some heavy metal pollutants in industrial wastewater could also be removed from wastewater via biological adsorption. However, due to high toxin concentration and generally low nitrogen and phosphorus concentration, microalgal growth rate are usually much lower in many industrial wastewater [78]. Thus, there is less potential for utilizing industrial wastewater as resource for large-scale microalgal biomass production.

Table 3
The classification and advantages/disadvantages of different microalgal isolation methods.

Isolation methods	Advantages	Disadvantages	Reference
Manual isolation methods			
Streak plate method	Relatively easy; low requirements on devices	Time-consuming; laborious; the species which cannot grow on solid substrate are excluded	–
Single-cell isolation by micropipette	Precision on the level of a single cell	Laborious; high requirements on experimental skills	[71]
Serial dilution technique	Relatively easy; low requirements on devices	Time-consuming; laborious	[67]
Micromanipulation	Precision on the level of a single cell	Laborious; high requirements on experimental skills and devices	[68]
Automatic isolation methods			
Atomized cell spray technique	Single cell can be obtained	Possible damage to cells; high requirements on devices	[67]
Flow cytometer	Precise and rapid; applicable directly to natural samples	High requirements on devices and its operation	[69,70]
Reinforcement techniques			
Immunological isolation technique	High specificity and selectivity	Possible damage to cells; high cost; complicated process	[71]
Non-immunological techniques	Applicable to sensitive cells; lower damage to cells	Lower specificity and selectivity	[71]

Table 4
Indices of microalgal species characterization for biomass/lipid production using wastewater as resource.

Index	Physical meanings and significance
Biomass production	
Maximal biomass production ($g\,L^{-1}$)	The maximal biomass concentration achieved in the cultivation process
Specific growth rate (μ, d^{-1})	The increment of per unit microalgal biomass per unit time
Doubling time (h)	The period of time required for a quantity to double in microalgal biomass
Biomass productivity ($g\,L^{-1}\,d^{-1}$)	The increment of microalgal biomass per unit time
Lipid production	
Lipid content (%)	The total lipid content in microalgal biomass
Triacylglycerol (TAG) content in lipid (%)	TAG is the direct material for the production of biodiesel. This index represents the direct material for biodiesel obtained from microalgal lipid
Fatty acid (FA) composition	FA composition means the degree of unsaturation and the carbon chain length of FA in microalgal biomass, and would affect the quality of biodiesel produced from microalgal lipid
Total fatty acid methyl ester (FAME) content (%)	FAME is the major constituent of biodiesel. This index represents the direct biodiesel production obtained from microalgal biomass
Lipid productivity ($mg\,L^{-1}\,d^{-1}$ or $mg\,m^{-2}\,d^{-1}$)	The increment of microalgal lipid per unit time
Pollutant removal	
Removal efficiency (%)	The removal efficiency of certain pollutant in certain time
Removal rate ($mg\,L^{-1}\,d^{-1}$)	The decrement of certain pollutant per unit time
Residual pollutant concentration ($mg\,L^{-1}$)	The residual concentration of certain pollutant after certain treatment time
Resistance to biotic pollution	
Resistance to bacteria	The competitive growth ability of microalgae against bacteria
Resistance to virus or fungus	The ability of microalgae to grow against parasitic virus or fungus
Resistance to predatory zooplankton	The ability of microalgae to survive the grazing of predatory zooplankton, and may relate to microalgal cell shape and size
Resource consumption	
Production and influence of Soluble Algal Products (SAP, $mg\,L^{-1}$)	SAP represents the total dissolved matters released by microalgal cell to the culture medium. SAP would contribute to the COD of microalgal culture medium, and influence the growth of microalgal cells
Minimal phosphorus content of microalgal cell (Q_0, %)	Q_0 is the minimal phosphorus content necessary for the metabolism of microalgal cell. It is the determining factor of potential biomass yield per phosphorus

Besides the final removal efficiency of certain pollutant, the removal rate and the residual pollutant concentration are also very important. The removal rate is directly related to the necessary hydraulic retention time (HRT) to achieve certain removal efficiency, and thus would influence the volume of structures and the operation process of wastewater treatment process. The residual pollutant concentration is an important index to evaluate whether a certain treatment process could achieve the wastewater discharge standard or not. In the aspect of nitrogen and phosphorus removal, microalgae could achieve much lower residual concentration than traditional treatment process. However, in the growth process microalgal cell would release some dissolved matters, which was defined as Soluble Algal Products (SAP) and would contribute significantly to the residual COD in culture medium [79]. Thus, the production of SAP would influence the lowest residual COD concentration achieved by microalgae significantly.

6.4. Indices about microalgal resistance to biotic pollution

A common and serious problem in the microalgal cultivation using unsterilized wastewater is that the microalgal biomass would crash unexpectedly after a period of cultivation time. This phenomenon happened frequently in some studies [5] and in the previous studies of the authors. Nevertheless, when wastewater was sterilized this phenomenon would hardly happen. Therefore, biotic inhibitory factors in wastewater, such as the pathogenic bacteria and virus or predatory zooplankton, were considered to be the most important reason for the crash of microalgal biomass [5]. Most of the data reviewed in section 4 was obtained under sterilized conditions.

However, in the large scale cultivation of microalgae using wastewater, the process of sterilization is very difficult and costly to conduct. It is also very difficult to prevent the cultivation system from biotic pollution in large scale [80]. Thus, the resistance to the

biotic inhibitory factors within wastewater is necessary to be considered in the screening of microalgal species adaptable to the growth in wastewater.

The contamination process and control approaches of these biotic inhibitory factors have been reviewed recently by Wang et al. [80]. Generally, the biotic contamination sources includes zooplankton, other microalgae, bacteria and virus. In the review of Wang et al., three control approaches were proposed, including filtration, the addition of chemical reagents and the change of cultivation conditions, such as light and temperature [80]. Besides the approaches mentioned above, ultraviolet (UV) disinfection is also one of the optional technologies to sterilize water, which is already used in wastewater treatment process. UV disinfection gains more and more attention because of its several advantages, including high disinfection efficiency with most viruses, bacteria and protozoa, no unidentified toxic disinfection byproducts (DBP) and safe operation [81]. However, the disinfection efficiency of UV lamps would decrease significantly with the increase of the turbidity of the culture, and a certain dose of UV irradiation of certain dose may also inhibit the growth of microalgal cell [82]. Therefore, the application of UV disinfection in microalgal cultivation needs more experimental verification.

The inhibitory effects of bacteria and virus on microalgae has been studied early on [83–85]. Some bacteria and viruses were also utilized to control harmful microalgae in algal bloom, such as *Sphin2 gomonas* sp. [86] and *Heterosigma akashiwo* virus clone 01 (HaV01) [87]. However, the report about microalgal abilities to resist pathogenic bacteria and virus are still very limited currently. It is still unclear which microalgal species or genus obtains higher resistance to bacteria, virus and fungus, and why they are resistant. More practical experiments are needed to identify proper microalgal species.

On the other hand, the resistance to predatory zooplankton could refer to some researches about the feeding properties of some predatory microorganism. Zhang et al. investigated the feeding characteristics of a golden alga (*Poterioochromonas* sp.) grazing on *Microcystis aeruginosa* [88] and other several microalgal species [89]. The relationship between the ingestion rate of *Poterioochromonas* sp. and the size/shape of feeding microalgal cells is summarized in Fig. 9. The microalgal cell with smaller size and more regular shape was much easier to be ingested by *Poterioochromonas* sp. These results suggested that the microalgal species with larger size and irregular shape (such as *Scenedesmus* sp.) or the microalgal species that could grow in cluster (such as *Botryococcus braunii*) may have stronger abilities to resist the grazing of zooplankton.

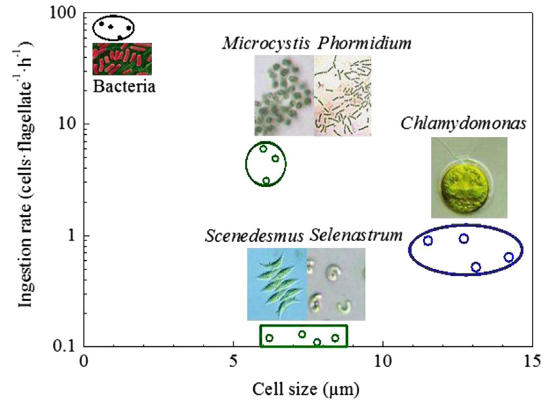


Fig. 9. The relationship between the ingestion rate of *Poterioochromonas* sp. and the size/shape of feeding microalgal cells.
(Adapted from the Ph.D. dissertation of Zhang [89]).

6.5. Indices related to resource consumption

SAP is the dissolved matter released by microalgal cell to the culture medium during the growth process [79]. A large part of this matters would remain in the water after the harvest of microalgal biomass. As water recycling was considered to be the most efficient way to reduce water consumption in microalgal cultivation [17], the accumulation of SAP in the repeated water recycling process would be foreseeable. The possible negative effects of SAP on the growth of certain microalgal species has been demonstrated by some researchers previously [90–93]. Thus, recently some researchers considered the production and effects on microalgal growth and lipid accumulation of SAP as an important index in the microalgal species screening [79]. The microalgal species with less SAP production or less negative effects caused by SAP seems to be more appropriate for the large-scale cultivation. Anyhow, more research is needed to identify the main inhibitory component in SAP, such as the research conducted by Zhang et al. [93] and develop efficient techniques to remove SAP from recycling water.

As for the phosphorus consumption in the production of microalgal biomass/lipid, Wu et al. proposed recently the possibilities to reduce phosphorus consumption via exploiting the abilities of microalgae growing with intracellular phosphorus [94]. The minimal phosphorus content of microalgal cell (Q_0) is an important parameter involved in microalgal growth process using intracellular phosphorus [95], and determines the potential biomass yield per phosphorus of a certain microalgal species [96]. The microalgal species with lower Q_0 would obtain higher potential of growing with intracellular phosphorus and higher potential biomass yield per phosphorus. In the previous study of the authors, *Scenedesmus* sp. LX1 was found to obtain the smallest Q_0 and the highest biomass yield of 6100 kg-biomass/kg-P among 7 tested microalgal species [96].

As reviewed above, in order to obtain an optimal microalgal species for biomass/lipid production using wastewater as resource in large-scale, a series of indices are needed, including the traditional screening indices as well as the indices specified for microalgal cultivation in wastewater under large scale. Up to now, no microalgal species has been demonstrated to meet all the requirements. Thus, the efforts on microalgal species isolation and characterization should be continued. On the other hand, the technologies to lower the requirements on microalgal species should also be developed, such as the techniques to prevent microalgal cultivation system from biotic contamination and to remove SAP from recycling water.

7. Limitations and future research needs for sustainable microalgal biomass/lipid production using wastewater as resource

7.1. Microalgal species for biomass/lipid production in unsterilized wastewater

As highlighted above, microalgal resistance to biotic pollution is necessary to be considered as an important index in the species screening for biomass/lipid production using wastewater as resource, because the existence of various bacteria, fungi and even zooplanktons was the most important characteristic of wastewater, and also one of the most significant difference between wastewater and artificial culture medium. Biotic contamination caused by these microorganisms was considered as one of the biggest challenges in the large scale cultivation of microalgae [5,80]. However, most of the data reported in recent studies was obtained under sterilized conditions. In laboratory scale, it is easy to sterilize wastewater as microalgal culture medium, and maintain axenic cultivation. But the cost and difficulty would increase exponentially in large-scale microalgal cultivation.

In order to guarantee stable microalgal biomass/lipid production using wastewater as culture medium against possible biotic

contamination, on one hand, it is necessary to develop the control techniques of biotic pollution as reviewed by Wang et al. [80]; on the other hand, it is also very important to select microalgal species with stronger resistance to biotic contamination, and demonstrate its performance on biomass/lipid production in unsterilized wastewater. The latter job has already been carried out by some researchers recently. In the study of Chinnasamy et al., 14 microalgal species were cultivated in the mixed wastewater containing 85–90% carpet industry effluents with 10–15% municipal wastewater, and the biomass and lipid production of these microalgal species in sterilized treated wastewater was compared with that in unsterilized wastewater [97]. It was found that *Botryococcus braunii*, *Chlorella saccharophila*, *Dunaliella tertiolecta* and *Pleurochrysis carterae* showed superior performance compared with other microalgal species both in sterilized and unsterilized wastewater. Among these four microalgal species, *Botryococcus braunii* and *Chlorella saccharophila* obtained almost the same biomass and lipid production in unsterilized wastewater as that in sterilized wastewater.

As highlighted in the study of Chinnasamy et al., in the future research it is necessary to demonstrate that the biomass and lipid production of certain microalgal species could be maintained over long cultivation periods against biotic pollution within wastewater. There is also the possibility that some novel techniques such as genetic manipulation of microalgal species, which was used to attempt to improve microalgal lipid content [98], could be utilized to increase microalgal resistance to biotic contamination.

7.2. Novel cultivation system for large-scale microalgal biomass production

The process of scaling up can be divided into two stages: (1) from the laboratory scale to the pilot scale/modest production plant scale; (2) from the pilot scale to industrial scale which would make a significant and sustainable contribution to global bioenergy production [99]. Most of the recent studies attempting to scale up were in stage (1). The cultivation scale of these studies was in the range from several liters to several cubic meters, which means in order to achieve microalgal biomass production under industrial scale it is necessary to enlarge the recent cultivation scale by tens of thousands of times. This, not surprisingly, would bring along a host of problems including political, social and economic as well as scientific [99]. The most important question is what kind of cultivation system could provide microalgal biomass production in such a huge scale economically and environmental-friendly. Due to the complexity of construction and the requirement of huge covering area, most of the photobioreactors and algal ponds used in recent studies are not suitable for industrial-scale microalgal biomass production. Therefore, the R & D efforts on novel cultivation system are very important in future research.

Recently, some novel cultivation systems were developed by some researchers. In the study of Christenson et al., a rotating algal biofilm reactor (RABR) was designed, built and tested under pilot scale (8000 l) in unsterilized municipal wastewater [100], and a biomass productivity of 20–31 $g\,m^{-2}\,d^{-1}$ was achieved, which was much higher compared with the productivity achieved in many other biofilm based cultivation systems. In this study, the cultivation and harvest process of microalgal biomass was integrated into one single system, and thus the problem involved in the process of biomass harvest was solved along with the process of cultivation. Similar design concept of combining cultivation and simplified harvest in one system was also utilized in other studies. Liu et al. developed a novel vertical plate attached microalgal photobioreactor [101], in which microalgae grew on the surface of vertical artificial supporting material to form algal film rather than in liquid culture medium. In this study, a biomass productivity of 50–80 $g\,m^{-2}\,d^{-1}$ was obtained outdoors for *Scenedesmus obliquus* using artificial culture medium. Zhuang et al. developed a novel suspended-solid phase photobioreactors (ssPBR) to improve microalgal biomass production and to simplify the harvest process of biomass [102].

All these efforts aimed to simplify biomass harvest process within cultivation system in situ. But there is also similar difficulty in scaling up these novel cultivation systems as others. The most important limitation was actually caused by light attenuation. According to Lambert–Beer law, the distance that the light on the surface could penetrate in microalgal culture was very limited [75]. Therefore, the depth of the algal pond was usually less than 0.5 m [103], leading to large requirement on land space. If the depth of microalgal pond could increase to 3–4 m, which is similar to the depth of common structures in wastewater treatment plant, the covering area of the whole system would decrease by almost one order of magnitude. Is this possible? An alternative question is “could microalgae grow without light?” According to the information reviewed above, many microalgal species did show the ability to use organic matters in wastewater for heterotrophic growth in recent studies, which means there is great potential to break through the limitation of light attenuation. In the future design of microalgal cultivation system, the potential of microalgae to grow using organic matters should be considered, demonstrated and utilized.

8. Conclusions

Wastewater has been considered as an important resource for economical and sustainable microalgal biomass/lipid production. However, due to the particularity of wastewater and huge scale of cultivation, microalgal species must meet higher requirements compared to traditional cultivation. Currently, most of the relevant researches are conducted in sterilized wastewater under laboratory scale or pilot scale. More efforts should be devoted to isolating microalgal species with superior performance and demonstrate its abilities to maintain stable biomass/lipid production against the biotic inhibitory factors within wastewater under large scale. Also, possible risk and adverse effects on biomass conversion processes induced by the cultivation in wastewater should be taken into consideration. Only in this way can renewable and sustainable microalgal biomass/lipid production using wastewater as resource be achieved.

Acknowledgments

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